High-dose erythropoietin has no effect on short- or long-term graft function following deceased donor kidney transplantation

Carsten Hafer[1,5] Thomas Becker[2,5], Jan T. Kielstein[1], Elisabeth Bahlmann[1], Anke Schwarz[1], Nikolai Grinzoff[1], Daria Drzymala[1], Isabelle Bonnard[1], Nicolas Richter[2], Frank Lehner[2], Juergen Klempnauer[2], Hermann Haller[1], Jana Traeder[3] and Danilo Fliser[4]

[1]*Department of Internal Medicine, Hannover Medical School, Hannover, Germany;* [2]*Department of Abdominal and Transplantation Surgery, Hannover Medical School, Hannover, Germany;* [3]*Department of Pathology, Hannover Medical School, Hannover, Germany and* [4]*Department of Internal Medicine, Saarland University Medical Centre, Homburg/Saar, Germany*

We evaluated short- and long-term effects of high-dose recombinant human erythropoietin (rHuEPO) in kidney transplantation in a prospective double-blind, placebo-controlled study. Patients with chronic kidney disease following receipt of a deceased donor kidney allograft were randomized to 3 doses of 40,000 units rHuEPO or placebo. The primary study end point was kidney function 6 weeks after transplantation with secondary end points of incidence of delayed graft function and kidney function 12 months after transplantation. Six weeks or 12 months after transplantation, the difference between estimated glomerular filtration rates was not significant comparing 44 patients who received rHuEPO to 44 patients receiving placebo. There was no significant difference regarding the incidence of delayed graft function (10 of 44 with rHuEPO compared with 14 of 44 on placebo). Protocol biopsies at 6 weeks and 6 months post transplant showed no significant differences in all assessed histological indices. The number and severity of adverse events were comparable between groups, as was patient and graft survival after 12 months. Thus, treatment with high-dose rHuEPO after kidney transplantation, although well tolerated, had no effect on long-term graft function or histology.

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Correspondence: *Danilo Fliser, Department of Internal Medicine, Saarland University Medical Centre, Kirrberger Strasse, 66424 Homburg/Saar, Germany. E-mail: indfli@uks.eu*

[5]These authors contributed equally to this work.

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In numerous studies, erythropoietin has revealed pleiotropic effects well beyond the maintenance of red blood cell mass.[1–3] In the embryo, erythropoietin is a major regulator of vascular formation and organ growth, and erythropoietin receptors are found in almost every embryonic tissue.[4] Erythropoietin receptors were found in many adult tissues including renal tissue, and even the notion of autocrine or paracrine erythropoietin systems has been raised.[5,6] Although the peritubular fibroblasts are the major adult site for erythropoietin production, erythropoietin receptors have been demonstrated also in other kidney cell types, e.g., proximal tubule epithelial cells, mesangial cells, and the glomerulus.[6] Moreover, erythropoietin has important cytoprotective effects on various cell lines and organs, and protection from ischemic injury and inhibition of apoptotic death–related pathways has been reported in the brain, heart, and renal tissue.[7–10] The intracellular pathways involved in these favorable erythropoietin effects may involve nuclear translocation of the transcription factor nuclear factor-κB, JAK2 phosphorylation, and phosphorylation of Akt (protein kinase B).[10,11]

An experimental study revealed that cobalt administration to rats caused upregulation of erythropoietin, and diminished the degree of renal injury caused by ischemia–reperfusion, suggesting that erythropoietin may also have an important role in renal ischemic preconditioning.[12] Indeed, subsequent studies from different laboratories demonstrated that preconditioning with recombinant human erythropoietin (rHuEPO) is protective against ischemia–reperfusion injury in rodents.[13–16] In this respect, data on specific protective effects of rHuEPO and its analogs on endothelial cells of glomeruli and tubulo-interstitial capillaries are of particular interest.[10] Furthermore, administration of rHuEPO may not only have protective effects on the vascular level but might also enhance the potential for regeneration, as we have demonstrated that erythropoietin stimulates proliferation and differentiation of functionally active endothelial progenitor cells in preclinical models.[17] These bone marrow-derived cells orchestrate endothelial and vascular repair mechanisms.

Renal ischemia, whether caused by shock or after surgery, is a major cause of acute kidney injury in men.[18,19] In this respect, kidney transplantation is a classical model of acute kidney injury due to ischemia–reperfusion injury, as the transplanted organ is connected to the recipients blood supply usually after several hours of 'cold ischemia'. Reperfusion, although essential for the survival of ischemic tissue, initiates a complex and interrelated sequence of events that result in injury and the eventual death of renal cells due to a combination of both apoptosis and necrosis.[20–22] Apoptotic cell death has been documented in human biopsies after renal ischemia–reperfusion, and inhibition of apoptotic signaling and cell death ameliorates the associated injury and inflammation in an experimental model of ischemic acute kidney injury.[22] Similarly, ischemia–reperfusion damage of transplanted kidney is thought to be a major factor limiting renal function after successful transplantation.[23,24] As long-term outcome is mainly predicted by graft function 12 months after successful transplantation, therapeutic regimens preventing ischemia–reperfusion injury and maintaining stable renal function are still needed.[25]

Administration of rHuEPO has been shown to reduce apoptosis in experimental models of acute and chronic renal ischemic injury. Thus, preconditioning and/or treatment with rHuEPO may also prevent renal tissue damage and loss of function as a result of ischemia–reperfusion injury after successful kidney transplantation. Despite these promising preclinical data, it is unclear weather rHuEPO treatment is also beneficial in patients after kidney transplantation. We therefore evaluated short-term and long-term effects of high-dose rHuEPO treatment in deceased donor kidney transplant recipients in a prospective double-blind, placebo-controlled study.

RESULTS

From the 90 patients enrolled and randomized to one of the two treatment arms, one patient in the rHuEPO arm did not receive active treatment because of a medical condition (dissection of the transplant artery before injection), and one patient in the placebo arm because of protocol violation (the study drug was not administered). All other patients received their assigned treatment (44 in the rHuEPO group and 44 in the placebo group). We analyzed data of these 88 patients on an intention-to-treat basis.

Baseline clinical characteristics of patients randomized to both treatment groups are presented in Table 1. They were comparable with respect to gender, age, body mass index, or pre-transplantation hemoglobin, as well as dialysis vintage. Mean estimated glomerular filtration rate (eGFR) was comparable in rHuEPO and placebo-treated patients 6 weeks, 6 months, and 12 months after transplantation (Figure 1 and Table 2). There was also no significant difference between groups with respect to the incidence of delayed graft function (10/44 patients rHuEPO vs. 14/44 patients with placebo).

Table 2 summarizes additional outcome data. Patient and graft survival showed no statistical difference over the study

Table 1 | Baseline clinical data of deceased donor kidney allograft recipients randomized to receive either rHuEPO or placebo treatment

	rHuEPO (n=44)	Placebo (n=44)	P-value
Gender (female/male)	19/25	18/26	0.8741
Age (years)	53.6 ± 1.8	49.8 ± 1.6	0.6770
Body mass index (kg/m^2)	25.3 ± 0.6	25.9 ± 0.6	0.8273
Dialysis vintage (months)	88.4 ± 5.1	67.6 ± 4.9	0.0651
Pre-transplant hemoglobin (g/dl)	12.7 ± 0.2	12.3 ± 0.2	0.0535
Pre-transplant hsCRP (mg/l)	6.3 ± 2.3	8.6 ± 2.2	0.4399
Cold ischemia time (min)	751 ± 38	807 ± 46	0.1876
HLA mismatches	2.7 ± 0.3	2.2 ± 0.3	0.7871

Abbreviations: HLA, human lymphocyte antigen; hsCRP, serum high-sensitivity C-reactive protein; rHuEPO, recombinant human erythropoietin.
Data are presented as mean ± s.e.m.

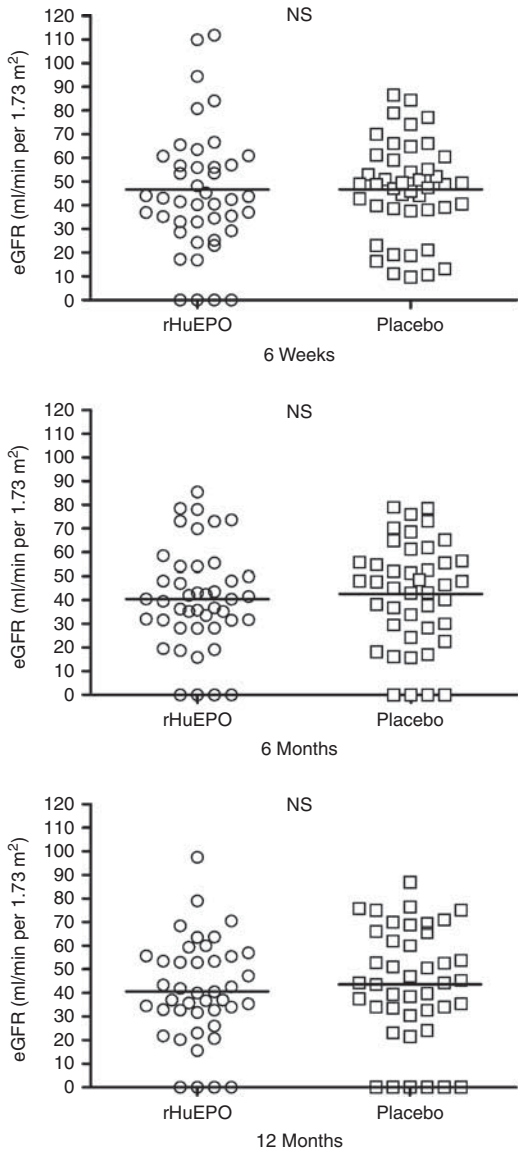

Figure 1 | Estimated glomerular filtration rate (eGFR; based on chronic kidney disease–Epidemiology Collaboration equation) in deceased donor kidney allograft recipients randomized to recombinant human erythropoietin (rHuEPO) or placebo 6 weeks, 6 months, and 12 months after transplantation. Non-survivors were excluded from the analysis. NS, not significant.

Table 2 | Outcome data of deceased donor kidney allograft recipients randomized to treatment with rHuEPO or placebo (intention-to-treat analysis)

	rHuEPO	Placebo	*P*-value	Difference of means (95% CI)
eGFR at 6 weeks (ml/min)	46.6 ± 4.3 (*n*=44)	46.8 ± 3.0 (*n*=44)	0.5097	−0.2 (−10.3 to 10.0)
eGFR at 6 months (ml/min)	40.4 ± 3.2 (*n*=44)	42.7 ± 3.4 (*n*=43)	0.4198	−2.3 (−11.5 to 7.0)
eGFR at 12 months (ml/min)	40.6 ± 3.3 (*n*=43)	43.6 ± 3.8 (*n*=42)	0.5561	−3.0 (−12.9 to 7.0)
Delayed graft function	10/44 (23%)	14/44 (32%)	0.6791	
Patient survival				
6 Weeks	44/44 (100%)	44/44 (100%)	0.9587	
6 Months	44/44 (100%)	43/44 (98%)	0.9139	
12 Months	43/44 (98%)	42/44 (95%)	0.9343	
Functioning grafts				
6 Weeks	40/44 (91%)	44/44 (100%)	0.7845	
6 Months	40/44 (91%)	40/44 (91%)	0.9731	
12 Months	39/43 (89%)	36/42 (82%)	0.8862	
Transfusions				
Patients	12 (27%)	15 (34%)	0.2612	
Blood packs	53	61	0.2188	
Hospitalization (days)	19.2 ± 11.5	22.4 ± 16.2	0.2962	

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; rHuEPO, recombinant human erythropoietin.
We found no significant differences between the two treatment groups. Data are presented as mean ± s.e.m.

observation period between groups. The cause of death is unknown for one patient in the rHuEPO treatment group (239 days after transplantation with a functioning graft). The causes of death in two patients of the placebo group were liver failure with consecutive multi-organ failure and dialysis dependency (146 days after transplantation) and sudden death (338 days after transplantation). Graft loss in the rHuEPO treatment group occurred because of renal venous thrombosis in two patients, insufficiency of renal artery anastomosis in one patient, and bend stenosis in another patient. In the placebo treatment group, graft loss occurred as a result of cholesterol embolism and renal infarction ($n=1$), renal artery thrombosis with consecutive renal infarction ($n=1$), severe pyelonephritis with interstitial fibrosis ($n=1$), and granulomatous interstitial nephritis with severe vascular rejection ($n=1$). The amount and severity of 'non-kidney' adverse events were similar between the two groups as well: small intestine perforation occurred in one patient assigned to the rHuEPO treatment group, whereas in the placebo treatment group subclavian vein thrombosis ($n=1$), ureteral necrosis ($n=1$), and bilateral pulmonary embolism ($n=1$) occurred.

In rHuEPO-treated patients, we observed a slightly but significantly higher mean hemoglobin level 2 and 4 weeks after transplantation (Figure 2), but 6 weeks after transplantation mean hemoglobin levels in both treatment groups were not significantly different. No patient needed conventional rHuEPO therapy within the first 6 months after successful kidney transplantation, and the number of blood transfusions in this period was comparable in both treatment arms (Table 2).

A total of 72 null biopsy specimens were available for examination (34 in the rHuEPO and 38 in the placebo group), whereas we obtained renal tissue specimens in 60 patients 6 weeks after transplantation (32 in the rHuEPO and 28 in the placebo group), and in 55 patients at 6 months after transplantation (31 in the rHuEPO and 24 in the placebo group). The missing biopsies were not performed either because of technical reasons or withdrawal of patients' consent for the biopsy. Histological assessment showed no significant differences in several parameters of acute and chronic allograft tissue changes at the respective time points, i.e., in null biopsies (Table 3), 6 weeks (Table 4) and 6 months after transplantation (Table 5). Similarly, the total number of biopsy-proven acute rejections in all protocol biopsies 6 weeks and 6 months after transplantation was not

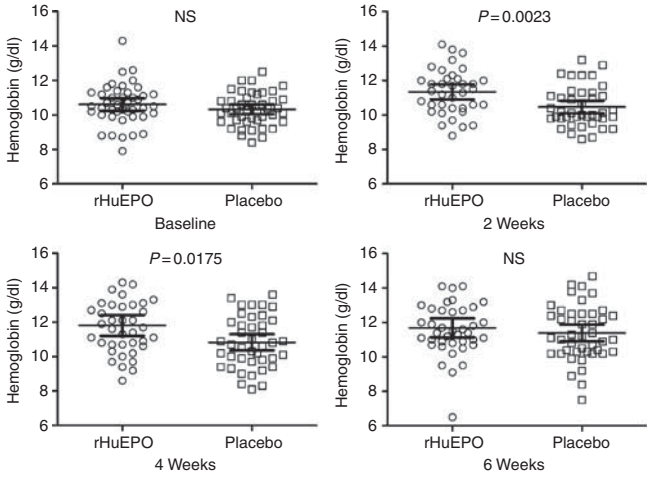

Figure 2 | Mean hemoglobin levels in deceased donor kidney allograft recipients randomized to recombinant human erythropoietin (rHuEPO) or placebo during the first 6 weeks after transplantation. NS, not significant.

Table 3 | Histological data from null biopsies of deceased donor kidney allografts in patients randomized to rHuEPO or placebo

	rHuEPO (n=34)	Placebo (n=38)
Acute tubular injury		
No	2	1
Focal	2	4
Diffuse	30	33
Interstitial fibrosis		
⩽10%	8	3
11–25%	0	0
>25%	0	0

Abbreviation: rHuEPO, recombinant human erythropoietin.
Data presented are from available biopsies. We found no significant differences between the two treatment groups.

Table 4 | Histological data from regular 6-week control biopsies of deceased donor kidney allografts in patients randomized to rHuEPO or placebo

	rHuEPO (n=32)	Placebo (n=28)
Acute tubular injury		
No	12	4
Focal	6	9
Diffuse	14	15
Interstitial fibrosis		
⩽10%	31	24
11–25%	1	3
>25%	0	1
BPAR	0	2

Abbreviations: BPAR, biopsy-proven acute rejection (including borderline rejection); rHuEPO, recombinant human erythropoietin.
Data presented are from available biopsies. We found no significant differences between the two treatment groups.

Table 5 | Histological data from regular 6-month control biopsies of deceased donor kidney allografts in patients randomized to rHuEPO or placebo

	rHuEPO (n=31)	Placebo (n=24)
Acute tubular injury		
No	8	7
Focal	9	5
Diffuse	14	12
Interstitial fibrosis		
⩽10%	24	16
11–25%	3	4
>25%	4	4
BPAR	7	9

Abbreviations: BPAR, biopsy-proven acute rejection (including borderline rejection); rHuEPO, recombinant human erythropoietin.
Data presented are from available biopsies. We found no significant differences between the two treatment groups.

significantly different between the two treatment arms; i.e., 7 in the rHuEPO group and 11 in the placebo group.

DISCUSSION

In the present prospective, double-blind, placebo-controlled trial, we evaluated short-term (6 weeks) and long-term (12 months) effects of high-dose rHuEPO in deceased donor kidney transplant recipients. The rationale for this study arose from findings of several experimental studies in which administration of rHuEPO was protective against ischemia–reperfusion injury in rodents.[13–16] In contrast to these promising experimental data, we did not observe a significant effect of rHuEPO on kidney transplant function, nor on the frequency of delayed graft function. Importantly, this clinical outcome was corroborated by the results of graft tissue histology obtained at predefined time points during our routine control biopsy program.[26,27] Here, we also found no significant differences between treatment groups with respect to acute and chronic renal tissue changes at an early (6 weeks) and late (6 months) time point. Furthermore, we have to emphasize that null biopsies were available, making the scoring and comparison of tissue injury between the two treatments even more robust. To our knowledge, this is the first controlled study in humans using high-dose erythropoietin treatment on different tissue injury parameters, e.g., acute tubular injury (ATI). As we observed rather large confidence intervals of the differences between groups, we cannot definitely exclude the possibility that in a much larger number of patients administration of rHuEPO might have had a positive effect on kidney graft function. However, from a practical, as well as economical, perspective, it is questionable whether a modest effect of rHuEPO therapy would justify such a labor-intensive and costly treatment in the clinical setting of kidney transplantation.

Our clinical outcome data are in agreement with those of Martinez *et al.*,[28] who studied high-dose rHuEPO in the setting of deceased donor kidney transplantation, and of Endre *et al.*,[29] who studied high-dose rHuEPO in the setting of acute kidney injury. In the French open-label multicenter randomized study,[28] the effect of high-dose epoetin-β administered during the first 2 weeks of renal transplantation was assessed in 104 patients at risk for delayed graft function. Epoetin-β (30,000 IU each) was administered before surgery and at 12 h, 7 days, and 14 days post transplantation. The control group received no treatment. At 1 month post transplant, the eGFR (Modification of Diet in Renal Disease (MDRD) Study formula) was 42.5 ± 19.0 ml/min in the epoetin-β group and 44.0 ± 16.3 ml/min in the control group; the difference was not significant. These data are almost identical to our finding on the eGFR 6 weeks after transplantation. In addition, the French authors did not find a significant difference in the frequency of delayed graft function (32.0% vs. 38.8%), which is also congruent with our results. Moreover, they found no difference in the incidence of serious adverse events, and, particularly, the number of venous or arterial thromboses was not increased.

In contrast to the French study, we now provide for the first time long-term data and histological assessment of the graft after high-dose rHuEPO treatment. Moreover, in contrast to the open-label design of the French study, we used the gold standard of clinical study designs, a double-blind, placebo-controlled study. In addition, we administered our first rHuEPO dose directly into the transplant artery

immediately before opening of the clamp in order to maximize a possible effect on reperfusion injury, whereas in the French trial all administrations were intravenous. Even with such a demanding approach we could not find an effect of rHuEPO on kidney graft function. In the other double-blind, placebo-controlled trial, the authors studied early treatment with rHuEPO on the development of acute kidney injury in 162 patients in two general intensive care units.[29] Patients received an intravenous dose of 500 U per kg body weight to a maximum dose of 50,000 IU. The primary outcome was the average plasma creatinine increase from baseline over a period of 4–7 days. The authors found no difference in the incidence of rHuEPO-specific adverse events, nor in the primary outcome between the placebo and treatment groups.

It is possible that the rHuEPO dose that we used in our study was too low to protect against ischemia–reperfusion injury after kidney transplantation. However, we administered a single rHuEPO dose of 40,000 IU, which corresponds to about 500 IU per kg body weight in an 80-kg patient, whereas the total rHuEPO dose administered in our study was 120,000 IU, which corresponds to about 1500 IU per kg body weight in an 80-kg patient. The doses used in different experimental models of acute kidney injury, including ischemia–reperfusion injury, ranged from 300 to 5000 IU per kg body weight and were thus comparable with the dose we used in the present study. Moreover, in their study, Sharples *et al.*[14] significantly reduced glomerular dysfunction and tubular injury in rats by administration of only 300 U rHuEPO per kg body weight. In contrast to the beneficial effect of rHuEPO administration on renal tissue histology in these animal experiments,[14,30] we observed no effect of rHuEPO even at a late time point 6 months after transplantation. It is doubtful whether a favorable effect of an acute rHuEPO treatment within the first 7 days post transplantation will unmask even later; i.e., years after transplantation. Another important inherent limitation of our study design in the clinical setting of organ transplantation is the timing of the intervention with rHuEPO in relation to the initiation of ischemia–reperfusion. With the intervention taking place after the harvesting of kidneys for transplantation, we are probably able to prevent reperfusion injury, but not ischemia, to the harvested organ to some extent. This point is important, as results of animal studies have indicated that high-dose rHuEPO treatment may be more efficient for prevention of ischemia than for prevention of reperfusion injury.[14,30]

Importantly, in all trials published so far, no significant safety problems even with high rHuEPO doses in clinical settings of acute kidney injury could be observed. This point is pertinent, as the use of such high doses has raised concerns regarding thrombosis, high blood pressure, and seizures. In the present study, we found no increase in adverse events as compared with the placebo treatment group despite a temporarily significantly higher mean hemoglobin level in the rHuEPO-treated patients—particularly, there was no evidence for increased intravascular thrombosis. Our primary end point was the difference in eGFR between treatment groups calculated after the chronic kidney disease-Epidemiology Collaboration equation, which is validated in the setting of kidney transplantation. However, even after calculation of eGFR using other equations such as the MDRD-based eGFR, or the measurement of cystatin C (data not shown), we did not observe a significant difference between the treatment groups at all time points studied. In addition, these robust clinical results are also supported by the histological findings. One limitation of the study is that the power analysis was based on the assumption that the standard deviation of the GFR would be below 20 ml/min. Hence, with the actually observed standard deviation slightly above this value, the study might not be adequately powered to exclude the possibility of a modest rHuEPO effect on graft function. Finally, we could speculate that a lack of erythropoietin receptor expression by tubular cells after ischemic tubular cell death could prevent efficacy of rHuEPO in this clinical setting. Thus, as already discussed above, preconditioning with high-dose rHuEPO before ischemic injury and cell death could be more effective in preventing tubular necrosis than administration immediately before reperfusion takes place.

Although we used high-dose rHuEPO treatment in the present study, the results obtained are nevertheless in line with those from large randomized controlled trials for correction of anemia in patients with chronic kidney disease, where no effect of long-term treatment with standard rHuEPO doses on the progression of kidney disease was documented.[31–33] Moreover, in the Trial to Reduce Cardiovascular Events with Aranesp Therapy study, the incidence of stroke was even significantly higher in the darbepoetin-α treatment cohort.[33] Finally, in recently published trials in patients with stroke and in patients with ST-segment elevation myocardial infarction, increased adverse effects rather than a benefit of high-dose rHuEPO therapy was observed.[34,35] The dialysis vintage tended to be different between the two groups despite the randomization procedure, which might have influenced the outcome.

Taken together, our results show that administration of rHuEPO was safe and well tolerated in deceased donor kidney allograft recipients, but without a significant effect on short-term and long-term outcome.

MATERIALS AND METHODS

Patients

Ninety dialysis patients undergoing deceased donor kidney transplantation were enrolled. Patients with preformed antibodies, i.e., highly immunized patients, were excluded from the study. Detailed information on enrollment of patients into the study is provided in the CONSORT flow diagram in Figure 3. All patients received a standard immunosuppressive regimen comprising interleukin-2 antibodies (20 mg basiliximab on day 0 and day 4), calcineurin inhibitors (cyclosporine A or tacrolimus), mycophenolate mofetil (1000 mg twice daily), and steroids (local tapering protocol, minimum dose 5 mg per day). The study population comprised patients aged 18–70 years with deceased donor kidney transplants.

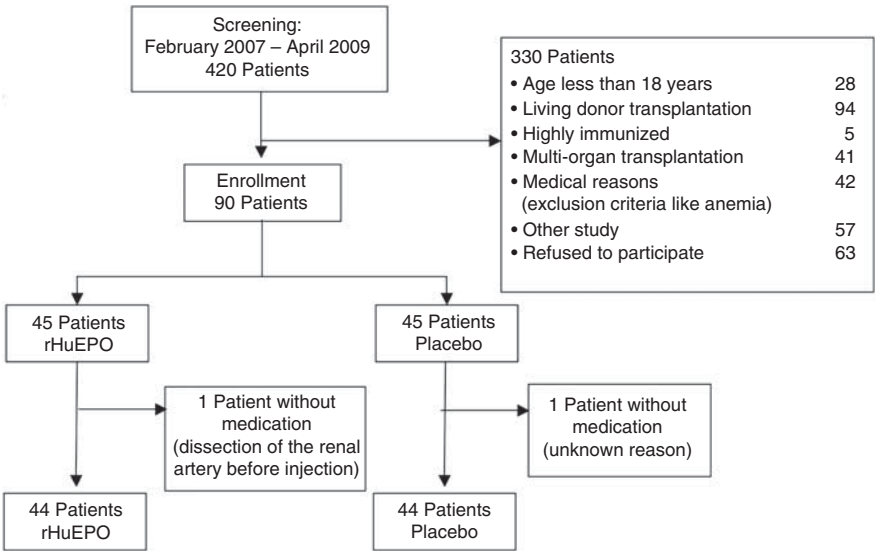

Figure 3 | CONSORT flow diagram on enrollment of patients into the study. Ninety patients undergoing deceased donor kidney transplantation were enrolled, and 88 were finally randomized to one of the two treatment arms; i.e., high-dose recombinant human erythropoietin (rHuEPO) or placebo.

Major specific exclusion criteria due to the rHuEPO treatment were significant cardiovascular events within 6 months before transplantation (e.g., myocardial infarction, stroke, transient ischemic attack, PRIND, thrombosis of large vessels), higher-grade occlusive disease of cerebral and/or peripheral arteries, any kind of hematological, bleeding, or thromboembolic disorders, acute or chronic inflammatory disorders (e.g., vasculitis, systemic lupus erythematodus), decompensated metabolic or liver diseases, uncontrolled hypertension, and a hemoglobin concentration of less than 8.0 g/dl and above 14.0 g/dl. Other key exclusion criteria included the following: recipients of a second transplant who experienced immunological graft loss of their first graft within 1 year, living donor grafts, cold ischemia time longer than 24 h, recipients of multiple organ transplants, recipients of ABO-incompatible transplants, patients with a historical peak panel-reactive antibody $>25\%$, patients with existing antibodies against human leukocyte antigens of the donor organ, and history of malignancy in the past 5 years.

Study design

The study was approved by the Ethics Committee of the Hannover Medical School. Appropriate reporting of the Ethics Committee approval to national and European regulatory bodies was made (EudraCT-number 2006-002938-38). We used a prospective double-blind, placebo-controlled, parallel group single-center study design. Recruitment started in February 2007 and was completed in May 2009. After written informed consent, all patients were randomly assigned to receive either 40,000 IU rHuEPO, i.e., epoetin-α, or placebo treatment. Both placebo- and verum (= rHuEPO)-containing vials were produced and provided by Janssen-Cilag GmbH (Neuss, Germany). Vials containing rHuEPO and those containing placebo had identical appearance and were thus indistinguishable for the investigators. They were identified only by the randomization number. The first intra-arterial injection of rHuEPO or placebo was administered already in the operation theater immediately before reperfusion directly into the iliac artery proximal to the anastomosis of the graft artery with clamped distal iliac artery in order to supply the full dose of rHuEPO into the reperfused kidney graft (after the vascular clamp was removed from the transplant renal artery). The second and third dose of 40,000 IU rHuEPO or placebo were administered intravenously on days 3 and 7 after successful transplantation. The rationale for the rHuEPO dose used and the number and timing of injections applied was primarily based on available experimental data,[13–16] and data from a recently published study with high-dose rHuEPO treatment in patients with stroke,[31] where a similar design and cumulative rHuEPO dose was used.

Red blood cell transfusions could have been given to patients in both study arms in order to maintain the hemoglobin level above 9.0 mg/l during the study period, whereas the hemoglobin concentration should not exceed 13.0 g/dl.

All relevant information with respect to the surgical intervention was recorded; i.e., duration of the intervention, duration of cold ischemia, blood pressure drops, and so on. We also assessed the frequency and duration of renal replacement therapy in each patient after transplantation and the individual need for blood transfusions. Furthermore, blood pressure was monitored and the co-medication recorded after transplantation on a regular basis. We assessed the following clinical and laboratory parameters before transplantation and at regular intervals thereafter: serum creatinine, eGFR based on the chronic kidney disease-Epidemiology Collaboration equation formula, endogenous creatinine clearance (when available), blood count, and inflammatory markers. Delayed graft function was defined as urine output of less than 500 ml in the first 24 h after transplantation and/or need of dialysis because of graft dysfunction within the first week after transplantation.

Last but not least, we also evaluated available data from renal histology examination in kidney specimens obtained during routine protocol biopsies (Hannover Medical School Transplant Biopsy Program) of kidney grafts before transplantation (null biopsies), as well as 6 weeks and 6 months after successful transplantation.[26,27] Examination of tissue specimens was performed in a blinded manner by one experienced pathologist. ATI was diagnosed if one or more of the following histological features were present: epithelial

swelling with lucency of the cytoplasm, loss of brush border, luminal dilatation with flattening of the epithelium, and cytoplasmatic vacuolization. ATI was semiquantitatively graded as mild when only a single tubule or a focal group of nephrons was involved, or as severe when at least two foci with several nephrons showed histological signs of ATI. No further sub-analysis of the localization of ATI (outer/inner cortex, specific nephron segments) was carried out. From all biopsies with sufficient material (comprising renal cortical tissue with at least one artery), the interstitial infiltrates were evaluated in a quantitative manner.

Statistical analysis

The primary study end point was allograft function 6 weeks after transplantation; i.e., chronic kidney disease-Epidemiology Collaboration equation-based eGFR. Secondary end points were incidence of delayed graft function and eGFR at 6 and 12 months after transplantation. Data from both treatment arms were compared using a *t*-test for random data. With a difference of at least 12 ml/min in eGFR between groups, the number of patients enrolled per group ($n = 45$) would allow the detection of a significant difference in eGFR between groups at a 5% level ($P < 0.05$) with a power of 80%. We also analyzed histology parameters in renal tissue specimens obtained before transplantation (null biopsies) and during regular control biopsies of kidney grafts 6 weeks and 6 months after transplantation. All data are shown as mean ± s.e.m., and 95% confidence interval where indicated. Confidence interval was calculated using Prism Graph version 5.0. (La Jolla, CA). As graft lost due to death that is unrelated to the treatment and should not confound the analysis of the treatment effect on eGFR, we have included only survivors in the analysis of the primary end point (graft function).

DISCLOSURE

All the authors declared no competing interests.

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